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ORIGINAL ARTICLE

Relationship between pregnancy, embryo development, and sperm deoxyribonucleic acid fragmentation dynamics

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Abstract The way the dynamics of DNA fragmentation affects the growth of embryos in real time, and effectiveness of infertility treatment using the ICSI procedure were determined in 148 couples treated with the ICSI technique. The percentage of sperm with fragmented DNA (known as the DNA fragmentation index [DFI]) in semen samples was determined at 3, 6 and 12 h. Embryo culture was assessed continuously during 12 h of observation monitoring.

Statistically significant difference was found in DFI at 12 h and outcome of treatment. For the remaining time intervals, no statistically significant differences were noted. An analysis of relationship between the DFI dynamics over time at individual measurements and achievement of pregnancy, confirmed a statistically significant relationship between the rate measured at 6–12 h of observations of DFI changes (DFI 12 h%/h), and achieving pregnancy. Correlation was observed between DFI (during 0, 3, 6 and 12 h), the growth rate in DFI, and time of embryo development. A statistically significant relationship was found between the rate from the start to the end of observations of the DFI, and outcome of treatment.

Intensity level regarding fragmentation of sperm DNA and its growth rate affected the time of embryo development in the ICSI procedure. The most significant prognostic factor for achieving pregnancy was intensification of sperm DNA fragmentation after 12 h.

1. Introduction

Obtaining pregnancy in the treatment procedure in vitro depends mainly on the quality of the embryo and oocyte. The reproductive potential of the sperm is conditioned not only by its morphologically normal structure and motility, but also sperm chromatin lesions to which belongs, among

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others, DNA fragmentation (Erenpreiss et al., 2006). It is known from the literature that the spermatozoon, even with considerably intensified DNA fragmentation, is able to fertilize an oocyte (Erenpreiss et al., 2006). The oocyte also possesses the ability to repair abnormal DNA; however, this depends on the type of damage, as well as the quality of the oocyte.

Sperm DNA fragmentation is not a static phenomenon, it is intensified in time and to various degrees, varies between individuals, and according to the external factors exerting an effect on sperm, related primarily with oxidative stress. The dynamics of DNA fragmentation may depend on the method of sperm preparation prior to its use for assisted reproductive technology (ART). The ultimate degree of DNA fragmentation also depends on the time elapsed from the collection of sperm until the use of a spermatozoon for ART procedures (Gosálvez et al., 2011b; Perrault et al., 2003; Evenson and Wixon, 2005; García-Contreras et al., 2011; Tremellen, 2008).

Therefore, it may be presumed that the quality of genetic material in sperm, which depends, among other things, on the level of intensity of nuclear DNA fragmentation, will exert a direct effect on the development of the embryo obtained as a result of the intracytoplasmic sperm injection (ICSI) procedure. For a long time, the quality of an embryo has been evaluated based on only morphological criteria, which seem to be insufficient to prognosticate the possibilities of achieving pregnancy after its transfer (Guerif et al., 2010; Arav et al., 2008). An alternative to morphological evaluation of an embryo is the observation of its development in real time due to the technology consisting in placing a camera inside the incubator (Arav et al., 2008; Cruz et al., 2011; Pribenszky et al., 2010; Wale and Gardner, 2010).

The objective of the presented study is to determine in what way the dynamics of DNA fragmentation affects the growth of embryos in real time, and the effectiveness of infertility treatment using the ICSI procedure.

2. Materials and methods

The study was conducted in 2012 and 2013, in the Non-Public Health Care Unit 'Ovum Reproduction and Andrology' in Lublin, and covered 148 couples treated due to infertility by the ICSI technique. The sperm DNA fragmentation index (DFI) was examined on the day of microinjection, as well as the dynamics of fragmentation (DFI-h), and retrospectively, the outcomes of treatment of the couples were evaluated: timing of embryo development and achievement of clinical pregnancy.

Female inclusion criteria were: age 25–35 years, FSH (follicle-stimulating hormone) <10 IU/ml and AMH (Anti-Mülerian Hormone) >1.5 ng/mL. Female exclusion criteria were: BMI (body mass index) <17 and >30, metabolic diseases. Male inclusion criteria were: age 25–35 years, treatment for infertility >1 year (couples had 4–6 prior intrauterine inseminations performed, males used dietary supplements in order to improve sperm parameters). Duration of the period preceding the treatment procedure was 1–4 years. Exclusion criteria for males were: severe asthenoteratozoospermia, symptoms of systemic diseases, inflammatory state of the reproductive organ, smoking BMI (Body Mass Index) <17 or >30, pre- and post-natal developmental disorders in reproductive organs, varices of the spermatic cord, taking medicines that may affect the quality and density of sperm up to 3 months prior to the study.

All patients signed a written consent to participate in the study. The study was approved by the Bioethics Commission.

In all patients, treatment with the ICSI procedure was applied using fresh oocytes and spermatozoa (sperm density $\geqslant 1$ million/ml). Sperm was obtained by masturbation three hours before microinjection and was examined directly after liquidation according to WHO criteria. Before the examination, males abstained from sex and alcohol for 3–4 days. In order to determine the percentage of sperm cells with fragmented DNA, the sperm chromatin dispersion test (SCD) was used, according to the instruction supplied with the kit (Dyn-Halosperm® kit, Halotech DNA SL, Madrid, Spain) (Fernández et al., 2005).The examination resulted in obtaining the sperm DFI – the percentage of sperm with DNA fragmentation. DFI was determined in sperm samples directly after liquidation (DFI 0 h), and subsequently after 3 h (DFI 3 h), 6 h (DFI 6 h), and 12 h (DFI 12 h) incubation in an automated incubator with 5% CO_2 at a temperature of 37 °C. On this basis, the dynamics over time between individual measurements was calculated (in percentages per hour).

DFI (3 h%/h) – is the % DFI per hour during the first 3 h, DFI (6 h%/h) – is% DFI per hour during the subsequent 3 h, and so on at individual intervals. Also, the rate of DFI increase was determined over the entire examination time from 0 to 12 h – DFI (%/h).

Ovarian stimulation was performed by the administration of gonadotropin-releasing hormone (GnRh) analog (Diphereline: Ipsen Pharma), followed by recombinant FSH (Gonal-F: Merck-Serono, Puregon: Organon) from cycle day 3 in a short protocol. The aspiration of oocytes was performed 36 h after the administration of recombinant HCG (r-hCG) (Ovitrelle: Merc-Serono).

After aspiration oocytes were placed in Fertilization medium (COOK, Sydney IVF, Australia) under mineral oil and after retrieval up to six oocytes (in accordance with Polish law) were subjected to ICSI procedure.

Oocytes were denuded from the granular layer and ICSI was performed 3 h after follicular puncture; fertilized cells were cultured in 25 µl drops of Cleavage medium (COOK, Sydney IVF, Australia) under mineral oil until day 2 (2–5 cell stage) in an automated incubator with 5% CO_2 at 37 °C fitted with time-lapse image acquisition (Time-lapse, Primo Vision EVO Microscope, Cryo-Innovation, Hungary). Fifty hours after ICSI, the culture media were changed to Blastocyst medium (COOK, Sydney IVF, Australia).

The growth of embryos was evaluated by monitoring at 10-min intervals using a camera placed inside the incubator. During the observation the embryos were not taken out of the incubator. Between image acquisitions the monitoring system was turned off to avoid exposure to electromagnetic radiation.

The 0 time was defined as the time of ICSI; the tF was defined as the time of the first frame in which the pronuclei were observed, while the tC as the time of the frame with the last observation. The stage of unicellular embryo after syngamy was defined as t1, and the subsequent stages were marked as t2, t3, t4, t5, t6, t7, t8. The time to the onset of formation of morula was defined as tM, while tB was the time in which a crescent-shaped area began to emerge from the

morula. The blastocysts were evaluated according to the criteria by the ASRM and ESHRE, and one of them was transferred to avoid multiple pregnancy.

At week 7 of pregnancy, embryonic echo and cardiac activity were assessed by ultrasound. The patients were divided into 2 groups: A – patients with pregnancy confirmed by ultrasonography (USG) at week 7, and B – patients in whom pregnancy was not achieved. In the group where the reproductive success was achieved 221 oocytes were subjected to the ICSI procedure and 88 embryos achieved the blastocyst stage, whereas in patients who were not pregnant out of 320 oocytes, subjected to ICSI, 110 attained the blastocyst stage. One embryo, which achieved the blastocyst stage at the earliest time, was transferred. The patients consciously decided about the transfer of singular blastocyst due to their partaking in the study. Blastocysts that were not transferred have been frozen.

In the presented study, the relationship is described between changes in the DFI at individual time intervals and the rate of DFI dynamics in time frames between individual measurements (in percentage per hour), and the outcome of treatment in the form of achieving pregnancy.

The results obtained were subjected to statistical analysis. The values of the parameters analyzed were presented as a mean value, minimum and maximum values, and standard deviation. The normality of distribution of variables in the groups examined was evaluated using the Shapiro–Wilk test. Differences between groups were investigated by nonparametric Manna–Whitney U test. The relationship between DFI parameters and individual times were tested by means of r–Pearson correlation. In order to determine the relationship between the achievement of pregnancy and intensification of DFI, the dynamics of DFI in individual time frames, and all times of embryo development, logistic regression analysis was applied. $p < 0.05$ was considered statistically significant. Statistical analysis was performed using Statistica 9.1 software (StatSoft, Poland).

3. Results

In both groups in the study, an intensification of DNA fragmentation was observed advancing over the time-lapse of incubation. This phenomenon had greater dynamics at the beginning of observations, i.e. within the first 3 h, while subsequently the rate of this process decreased.

In the group where the reproductive success was achieved at the beginning of observations the mean DFI value was 14.5, whereas in patients who did not achieve pregnancy – 16.32. Subsequent DFI measurements were recorded at 3, 6 and 12 h, and in the group with pregnancy achieved an increase in DFI up to the value 42.93, then to 51.96, and finally to 64.98. While observing this phenomenon in the group of patients who were not successful in reproduction higher values were noted in analogous time frames, i.e. 43.72, followed by 52.73, and ultimately 72.65. While comparing DFI values in individual measurement times between the groups of patients who achieved pregnancy and those who did not, a statistically significant difference between groups was found in the DFI value after 12 h ($Z = -3.499$, $p = 0.0005$; Z – Mann Whitney's test result; p – statistical significance), while with respect to the remaining values no statistically significant differences were noted (Fig. 1).

Based on the DFI values obtained in individual measurement times, the growth rate of the fragmentation phenomenon was calculated in percentages per hour. Initially, within the first 3 h, this rate was 14.31%/h in the group of patients with pregnancy, and 14.57%/h in the remainder. In the subsequent time frame, the dynamics of the phenomenon decreased down to 3.01%/h, and finally to 2.17%/h in the group with achieved pregnancy. The rate calculated in analogous times in the group with no reproductive success was 3.00%/h, respectively, and 3.32%/h, finally.

While comparing the dynamics of intensification of DNA fragmentation between the groups examined, statistically

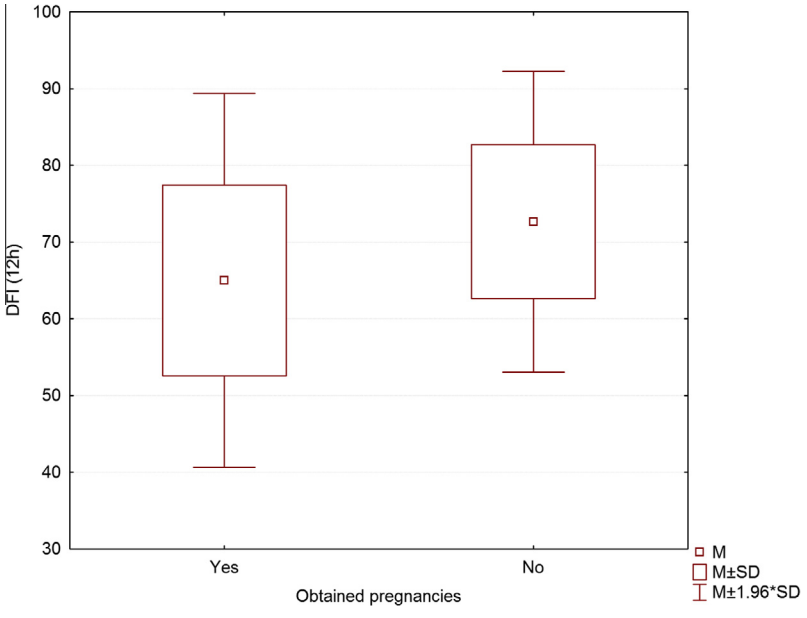

Figure 1 DFI after 12 h in groups with and without achieved pregnancy, $Z = -3.499$, $p = 0.0005$.

significant differences were found between the groups in the study according to the rates registered at 6 and 12 h, ($Z = -3.844$, $p = 0.0001$), and between 0 and 12 h ($Z = -2.959$, $p = 0.003$). In the case of the remaining measurements, no statistically significant differences were confirmed (Fig. 2).

During the observation of embryos, the times of their growth were measured until the obtaining of individual developmental stages, and a statistically significant difference was found between groups according to the value of time which elapsed from microinjection to the obtaining of the stage of four-cell embryo – t4 ($Z = -2.518$, $p = 0.012$). In the group of patients who had a reproductive success the embryos achieved this developmental stage more quickly than in the group without pregnancy. The comparison of times within which the embryos achieved other developmental stages did not confirm any statistically significant differences between the groups examined (Fig. 3).

While analyzing the effect of intensification of sperm DNA fragmentation on the dynamics of embryos development in patients in individual groups, the presence of statistically significant positive correlation was observed between the initial DFI value, and the times of embryo growth from the moment of occurrence of the pronuclei to the five-cell stage. Low values of the initial DFI of spermatozoa used for the ICSI were also related with an acceleration of obtaining the blastocyst stage by the embryo. In the case of DFI examined after 3 h of sperm incubation, in both groups, a positive correlation was

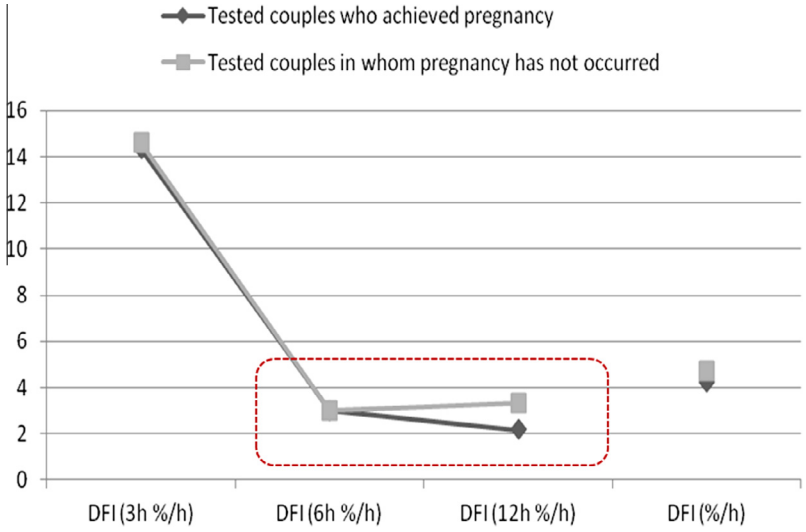

Figure 2 Rate of DFI growth rate in individual measurement times (*x*h%/h) in groups where pregnancy was achieved and where pregnancy did not occur.

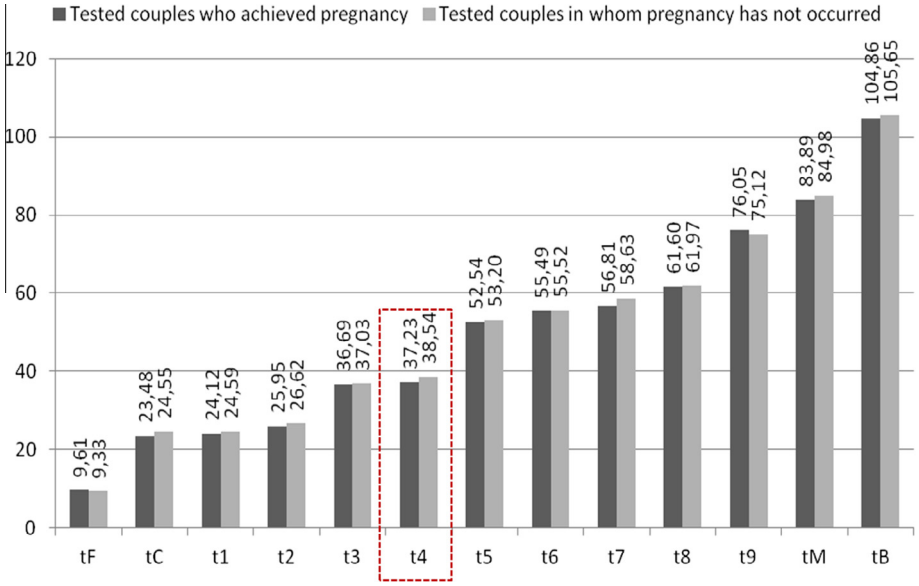

Figure 3 Dynamics of development of an embryo at individual measurement times (*t*) in groups where pregnancy was achieved and where pregnancy did not occur.

confirmed with the time of disappearance of the pronuclei and 2-cell stage of the embryo, and in the group with achieved pregnancy, also with 5- and 6-cell embryo. DNA fragmentation which occurred after 12 h was positively correlated only with the growth time of embryos from the moment of disappearance of the pronuclei until the obtaining of division into 3 cells, and also with the t5 in the group with achieved pregnancy, while in the remaining patients no significant relationships were observed (Table 1).

While analyzing the effect of the dynamics of DNA fragmentation on the rate of embryos development in both groups, it was noted that the rate of DFI growth within the first 3 h negatively correlated with the time when the pronuclei disappear and 2-cell stage, and with t5 and t6 times in the group with achieved pregnancy. The dynamics of DNA fragmentation between 3 and 6 h incubation did not affect the achievement of developmental stages by embryos in women with achieved pregnancy, whereas in the remainder a negative correlation was observed only with the times tc and t1. The rate of fragmentation noted after 6 h in each group was positively correlated with the times of embryo development from the stage of disappearance of pronuclei to the blastocyst time, and in the group with achieved pregnancy, also with the times of achieving subsequent stages to the 3-cell stage.

Analysis of the total rate of DFI growth from the beginning of measurement until 12 h incubation (DFI%/h) showed lack of its correlation with the times of development of embryonic stages in women with achieved pregnancy, while in the group with no reproductive success, a statistically significant positive correlation was found between DFI%/h and the dynamics of embryonic development from the stage of emergence of pronuclei to the obtaining of 5 blastomeres, as well as with the time of achieving the stage of a blastocyst (Table 2).

In order to discover whether the achievement of the goal which is the obtaining of pregnancy is affected jointly by all the variables analyzed, the logistic regression model was applied. The dependent variable was the probability of achieving pregnancy, and the independent variables tested were the intensity of DFI, and dynamics of DFI in individual time intervals and all times of embryo development. Ultimately, only one variable was left in the model – intensity of DNA fragmentation after 12 h as the only one statistically significant.

Results of the analysis showed that together with an increase in the value of the variable DFI 12 h by one unit, the chance of conception is lower by 5.95% (Table 3).

Table 1 Relationship between the DFI (DNA fragmentation index) parameter (at times 0, 3, 6 and 12 h), and times of embryo development in groups with and without achieved pregnancy.

Time		Patients with achieved pregnancy				Patients without achieved pregnancy			
		DFI (0 h)	DFI (3 h)	DFI (6 h)	DFI (12 h)	DFI (0 h)	DFI (3 h)	DFI (6 h)	DFI (12 h)
tF	*r*	**0.333**	−0.009	−0.069	−0.008	**0.495**	−0.004	−0.012	0.180
	p	**0.014**	0.958	0.621	0.954	**0.000**	0.966	0.910	0.083
tC	*r*	**0.488**	**0.349**	0.263	**0.383**	**0.437**	**0.303**	0.008	0.064
	p	**0.000**	**0.010**	0.054	**0.004**	**0.000**	**0.003**	0.942	0.541
t1	*r*	**0.527**	0.257	0.211	**0.357**	**0.607**	0.062	−0.175	0.038
	p	**0.000**	0.061	0.126	**0.008**	**0.000**	0.553	0.092	0.715
t2	*r*	**0.606**	**0.292**	0.202	**0.336**	**0.625**	**0.251**	0.110	0.125
	p	**0.000**	**0.032**	0.144	**0.013**	**0.000**	**0.015**	0.291	0.230
t3	*r*	**0.470**	0.169	0.128	**0.281**	**0.391**	0.103	−0.107	0.016
	p	**0.000**	0.223	0.356	**0.040**	**0.000**	0.322	0.305	0.879
t4	*r*	**0.599**	0.179	0.210	0.223	**0.632**	0.175	−0.014	−0.039
	p	**0.000**	0.195	0.127	0.105	**0.000**	0.092	0.895	0.711
t5	*r*	**0.299**	**0.337**	0.239	**0.334**	**0.255**	−0.056	−0.040	−0.057
	p	**0.028**	**0.013**	0.082	**0.014**	**0.013**	0.591	0.701	0.583
t6	*r*	0.100	**0.294**	0.037	0.063	−0.016	−0.086	−0.101	−0.044
	p	0.471	**0.031**	0.791	0.653	0.875	0.409	0.332	0.673
t7	*r*	0.026	0.064	−0.095	−0.052	−0.075	0.050	−0.040	−0.061
	p	0.849	0.648	0.497	0.710	0.469	0.633	0.702	0.559
t8	*r*	0.008	0.040	−0.107	−0.074	−0.071	−0.154	**−0.210**	0.083
	p	0.952	0.772	0.441	0.596	0.496	0.139	**0.042**	0.426
t9	*r*	0.078	−0.036	0.186	0.077	0.107	0.114	0.105	0.071
	p	0.576	0.795	0.177	0.579	0.304	0.273	0.315	0.496
tM	*r*	−0.098	−0.113	0.053	−0.094	−0.130	−0.059	0.042	0.011
	p	0.481	0.416	0.702	0.501	0.212	0.574	0.691	0.915
tB	*r*	**0.511**	−0.069	0.092	0.188	**0.508**	0.143	0.072	0.104
	p	**0.000**	0.618	0.508	0.173	**0.000**	0.171	0.490	0.318

*Statistically significant correlations written in bold letters.

p – statistical significance; *r* – correlation rate; tF – time of the first frame in which both pronuclei could be observed; tC – the frame with the last observation of both pronuclei; t1 – time for the corresponding number of 1 cell; t2 – time for the corresponding number of 2 cells; t3 – time for the corresponding number of 3 cells; t4 – time for the corresponding number of 4 cells; t5 – time for the corresponding number of 5 cells; t6 – time for the corresponding number of 6 cells; t7 – time for the corresponding number of 7 cells; t8 – time for the corresponding number of 8 cells; t9 – time for the corresponding number of 9 cells; tM – the first frame in which the embryos were compacting into the morula stage; tB – the frame in which a crescent-shaped area began to emerge from the morula.

Table 2 Relationship between DFI (DNA fragmentation index) growth rate (3 h%/h, 6 h%/h, 12 h%/h and %/h) and times of embryo development in groups of patients with and without achieved pregnancy.

Time		Patients with achieved pregnancy				Patients without achieved pregnancy			
		DFI (3 h%/h)	DFI (6 h%/h)	DFI (12 h%/h)	DFI (%/h)	DFI (3 h%/h)	DFI (6 h%/h)	DFI (12 h%/h)	DFI (%/h)
tF	*r*	−0.0074	−0.0656	0.0437	−0.1860	−0.0044	−0.0082	0.1777	−0.1787
	p	0.958	0.637	0.754	0.178	0.966	0.937	0.087	0.085
tC	*r*	**0.3498**	0.0053	**0.3095**	0.1686	**0.3033**	**−0.2453**	0.0548	**−0.2391**
	p	**0.010**	0.970	**0.023**	0.223	**0.003**	**0.017**	0.600	**0.020**
t1	*r*	0.2570	0.0219	**0.3165**	0.1191	0.0619	**−0.2273**	0.1581	**−0.3753**
	p	0.061	0.875	**0.020**	0.391	0.553	**0.028**	0.128	**0.000**
t2	*r*	**0.2922**	−0.0146	**0.2955**	0.0536	**0.2514**	−0.0991	0.0409	**−0.3128**
	p	**0.032**	0.917	**0.030**	0.701	**0.015**	0.342	0.696	**0.002**
t3	*r*	0.1686	0.0036	**0.2788**	0.0638	0.1033	−0.1937	0.0898	**−0.2492**
	p	0.223	0.979	**0.041**	0.647	0.322	0.061	0.390	**0.015**
t4	*r*	0.1791	0.0805	0.1350	−0.0700	0.1749	−0.1598	−0.0269	**−0.4578**
	p	0.195	0.563	0.331	0.615	0.092	0.124	0.797	**0.000**
t5	*r*	**0.3367**	−0.0104	0.2625	0.2138	−0.0561	0.0065	−0.0260	**−0.2209**
	p	**0.013**	0.941	0.055	0.121	0.591	0.951	0.803	**0.032**
t6	*r*	**0.2945**	−0.1867	0.0555	0.0167	−0.0861	−0.0299	0.0292	−0.0267
	p	**0.031**	0.176	0.690	0.905	0.409	0.775	0.780	0.798
t7	*r*	0.0636	−0.1464	0.0049	−0.0720	0.0499	−0.0818	−0.0295	−0.0015
	p	0.648	0.291	0.972	0.605	0.633	0.433	0.778	0.988
t8	*r*	0.0404	−0.1417	−0.0149	−0.0870	−0.1538	−0.0826	**0.2250**	0.1191
	p	0.772	0.307	0.915	0.532	0.139	0.428	**0.029**	0.253
t9	*r*	−0.0362	0.2207	−0.0436	0.0449	0.1142	0.0100	−0.0063	−0.0110
	p	0.795	0.109	0.754	0.747	0.273	0.923	0.952	0.916
tM	*r*	−0.1129	0.1415	−0.1691	−0.0525	−0.0587	0.0907	−0.0185	0.0968
	p	0.416	0.308	0.221	0.706	0.574	0.385	0.859	0.353
tB	*r*	−0.0695	0.1484	0.1816	−0.0618	0.1425	−0.0465	0.0477	**−0.2521**
	p	0.618	0.284	0.189	0.657	0.171	0.656	0.648	**0.014**

*Statistically significant correlations written in bold letters.
p – statistical significance; *r* – correlation rate; tF – time of the first frame in which both pronuclei could be observed; tC – the frame with the last observation of both pronuclei; t1 – time for the corresponding number of 1 cell; t2 – time for the corresponding number of 2 cells; t3 – time for the corresponding number of 3 cells; t4 – time for the corresponding number of 4 cells; t5 – time for the corresponding number of 5 cells; t6 – time for the corresponding number of 6 cells; t7 – time for the corresponding number of 7 cells; t8 – time for the corresponding number of 8 cells; t9 – time for the corresponding number of 9 cells; tM – the first frame in which the embryos were compacting into the morula stage; tB – the frame in which a crescent-shaped area began to emerge from the morula.

Table 3 Parameters of logistic regression model.

$Chi^2 = 15.651$; $p = 0.00008$	
	DFI (12 h)
Logistic regression model coefficient	−0.0613
p level	0.0002
Odds ratio	0.9405
[95% confidence interval]	[0.9107; 0.9713]

DFI – DNA fragmentation index; *p* – statistical significance.

4. Discussion

Based on the results of the study it was confirmed that in the case of sperm chromatin lesion the most important prognostic factor in the achievement of pregnancy in the ICSI procedure is the intensity of sperm DNA fragmentation after 12 h. In international literature, there are many reports concerning disorders in the integrity of sperm DNA chromatin on the effectiveness of infertility treatment by the methods of assisted reproductive technology, which are frequently unequivocal.

Gosálvez et al. (2011b) examined fresh and frozen-thawed sperm from 5 donors with confirmed fertility. In their study, the researchers measured the rate of DFI increase within the first 6 h of incubation, and obtained the value of 1.6% per hour for fresh sperm, and 4.3% per hour for thawed sperm. In the presented study, 8.66%/h of fragmentation increase, on average, was obtained within the first 6 h of incubation in the group with reproductive success, and 8.78%/h in the remainder. These discrepancies may result from the fact that in this study participated patients treated due to infertility, while Gosálvez et al. (2011b) examined individuals possessing offspring. In other studies, Gosálvez et al. (2011a) confirmed an increased rate of DNA fragmentation in the group of 10 infertile males, compared to sperm donors with confirmed fertility. The mean rate of DFI increase among males treated for infertility was 10.13% per hour within 4 h, this result being close to the result obtained in the presented study (Gosálvez et al., 2011a).

The reasons for which the dynamics of DNA fragmentation does not differ individually have not been fully explained. García-Peiró et al. (2011) showed that the factor which accelerates an increase in DFI rate is the presence of varices of the spermatic cord, and explained it by the concept of oxidative

stress accompanying this disease. Other studies carried out by Santiso et al. (2012) also seem to confirm the effect of oxidative stress on the generation of changes in DFI over time. These researchers investigated the effects of agents that cause genetic damage (ionizing radiation; elevated temperature; acidic pH and the nitric oxide (NO) donor sodium nitroprusside [SNP]). All agents, with the exception of ionizing radiation, accelerated DNA fragmentation kinetics following chronic exposure. Transient exposure to NO and heat, but not acidic pH, increased the basal level of DFI. Despite the removal of the 3 toxicants, the remaining sperm, following acute exposure, showed a decrease in their expected DNA longevity. The researchers explained the effect of hyperthermia, the nitric oxide donor sodium nitroprusside on increased DNA fragmentation by the concept of oxidative stress (Santiso et al., 2012).

Other studies evaluating therapy of male infertility with anti-oxidants, conducted by Abad et al. (2013) among 20 patients, also drew attention to the importance of oxidative stress on the generation of sperm DNA lesions. The studies indicated a relationship similar to that observed in the presented study, between the dynamics of DNA fragmentation and the number of achieved pregnancies during infertility treatment.

Factors responsible for the damage of sperm DNA may exert an effect not only on chromatin, but most probably also contribute to the decrease in the semen parameters evaluated by microscopic analysis. The significant relationship between the effectiveness of IVF procedure and sperm quality was confirmed in the studies by Jędrzejczak et al. (2004). This indirectly confirms the results of the presented study, because an intensified DNA fragmentation is correlated with abnormal sperm parameters, as confirmed by the studies by Fei et al. (2013). Dobrzyńska et al. (2010) also noted the relationship between the intensification of DNA fragmentation and reduced percentage of sperm with progressive motility.

Studies conducted by Pregl et al. (2013) showed a negative correlation between the intensification of DNA fragmentation and the achievement of pregnancy and the quality of embryos in the conventional IVF. Similar results were obtained by Jiang et al. who analyzed in their studies the effect of DFI on the percentage of biochemical pregnancies achieved in the ICSI procedure (Jiang et al., 2011). In turn, Sadeghi et al. (2011) did not find any relationship between the intensification of sperm DNA fragmentation and the effectiveness of IVF. Rougier et al. (2013) observed that the method of sperm preparation before ICSI has an effect on the dynamics of DNA fragmentation, which may explain the discrepancies obtained by the researchers.

In their studies, Gawecka et al. (2013) induced chromatin damage in mouse sperm, and subsequently used them for the ICSI procedure. They observed the prolongation of the initial stages of embryo development together with an increase in the intensification of sperm DNA fragmentation, which is consistent with the results of the presented study. They discovered an interesting fact, that in the case of injection of sperm with considerable chromatin damage, a delay is noted in the development of paternal prenuclei, compared to maternal prenuclei, which could suggest the prolongation of the period of DNA repair in the oocyte. Thus, while analyzing the results by Gawecka et al. (2013) it may be expected that the time of sperm DNA repair may vary and depends on its intensity, which may explain the effect of the dynamics of fragmentation on the times of embryo development observed in this study.

Similar conclusions concerning the effect of sperm chromatin lesions were drawn by Simon et al. (2014) who examined 215 men from infertile couples undergoing assisted reproduction techniques, and discovered that increased sperm DNA damage adversely affects embryo quality starting at day 2 of early embryonic development, and continuing after embryo transfer, resulting in reduced implantation rates and pregnancy outcomes. The paternal effect of sperm DNA damage was observed at each stage of early embryonic development. In their study, the paternal influence of damaged chromatin was more prominent after zygotic transcriptional activation. The prolonged paternal effect on the developing embryo may be due to the active repair mechanism present in oocytes that tends to overcome the damaged paternal chromatin. The probability of eliminating an embryo fertilized by a sperm with damaged DNA is higher at the blastocyst stage than the cleavage stage; therefore, blastocyst transfer could be recommended for better implantation success. Finally, they recommend ICSI treatment for patients with a higher percentage of sperm with DNA damage (Simon et al., 2014).

It may be presumed that the progressing phenomenon of chromatin fragmentation is conditioned with the processes which have not yet been investigated, and are probably related with a disturbance in the balance of the oxidoreductive system. Probably, this phenomenon does not exclusively concern the sole fragmentation, but perhaps other DNA damage. It is known that some processes leading to damage of the genetic material, such as methylation, are also of a dynamic character. Shortly after penetration of the oocyte, sperm DNA is actively demethylated, which is required for totipotent zygotic development. Aberrant DNA methylation is thought to be associated with altered chromatin condensation of spermatozoa. This is confirmed by the studies conducted on an animal model by Rahman et al. (2014). The objective of their study was to investigate the dynamics of DNA methylation reprogramming in the paternal pronucleus, and subsequent fertilization potential of heat-stressed bull spermatozoa having altered chromatin condensation. Hence, bovine zygotes (n = 1239) were collected at 12, 18 and 24 h post insemination (hpi) and stained with an antibody against 5-methylcytosine. Fluorescence intensities of paternal and maternal pronuclei were measured by ImageJ. DNA methylation patterns in paternal pronuclei derived from heat-stressed spermatozoa did not differ between time points, whereas control zygotes clearly showed demethylation and de novo methylation at 18 and 24 hpi, respectively. Moreover, heat-stressed spermatozoa showed a highly reduced fertilization rate compared with non-heat-stressed or normal control spermatozoa. Their data showed that the normal pattern of active DNA demethylation, followed by de novo methylation in the paternal pronucleus, was perturbed when oocytes were fertilized with heat-stressed spermatozoa, which might be responsible for decreased fertilization potential.

The presented study is probably the first to evaluate the effect of the dynamics of DNA fragmentation on the development of human embryos in real time, and the effectiveness of the ICSI procedure in humans. The phenomenon of increased sperm DNA fragmentation over time should incline the team supervising the performance of the ICSI procedure toward striving in their daily practice for possibly the closest moment

in time of sperm collection and its use for assisted reproductive techniques. In the presented study, the sperm was collected 3 h before microinjection, with a 3–4 day sexual and alcohol abstinence; however, in daily practice, the time interval between sperm collection and its preparation before fertilization may differ, according to individual situations. It is known that sperm donation takes the patient various amounts of time, while microinjection must be performed at a precisely specified moment. Thus, the final determination of DNA fragmentation will be affected by: the patient's sexual abstinence, method of sperm preparation, and time which elapsed between sperm donation and its use for ART.

The presented study is an attempt to emphasize the importance of the dynamics of DNA fragmentation in human reproductive processes, and will certainly require continuation in the future.

5. Conclusions

1. Dynamics of embryo development until the moment of obtaining 5-cell stage, as well as the time of emergence of a blastocyst, depends on the initial sperm DNA fragmentation.
2. Intensification of sperm DNA fragmentation within the first 3 h affects the rate of embryo development to the 2-cell stage.
3. The most important prognostic factor in the achievement of pregnancy is intensification of sperm DNA fragmentation after 12 h.

Authors' contribution

Artur Wdowiak:study conception and design, data collecting and interpretation, literature review, writing the manuscript.

Iwona Bojar:study conception, final acceptance.

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Ethics statement

The study protocol was approved by the Institutional Review Board (IRB) of the Bioethics Committee at the Institute of Rural Health in Lublin (IRB No. 24/2013). Informed consent was confirmed (or waived) by the IRB.

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References

Abad, C., Amengual, M.J., Gosálvez, J., Coward, K., Hannaoui, N., Benet, J., García-Peiró, A., Prats, J., 2013. Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. Andrologia 45, 211–216.

Arav, A., Aroyo, A., Yavin, S., Roth, Z., 2008. Prediction of embryonic developmental competence by time-lapse observation and 'shortest-half' analysis. Reprod. Biomed. Online 17, 669–675.

Cruz, M., Gadea, B., Garrido, N., Pedersen, K.S., Martinez, M., Perez-Cano, I., Muñoz, M., Meseguer, M., 2011. Embryo quality. blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. J. Assist. Reprod. Genet. 28, 569–573.

Dobrzyńska, M.M., Tyrkiel, E., Derezińska, E., Ludwicki, J., 2010. Is concentration and motility of male gametes related to DNA damage measured by comet assay? Ann. Agric. Environ. Med. 17, 73–77.

Erenpreiss, J., Spano, M., Erenpreisa, J., Bungum, M., Giwercman, A., 2006. Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J. Androl. 8, 11–29.

Evenson, D.P., Wixon, R., 2005. Environmental toxicants cause sperm DNA fragmentation as detected by the Sperm Chromatin Structure Assay (SCSA®). Toxicol. Appl. Pharmacol. 207, 532–537.

Fei, Q., Jin, J., Ni, W., Huang, X., 2013. Variation of sperm DNA fragmentation index in male partners from infertile couples. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 30, 357–361.

Fernández, J.L., Muriel, L., Goyanes, V., Segrelles, E., Gosálvez, J., Enciso, M., LaFromboise, M., De Jonge, C., 2005. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion (SCD) test. Fertil. Steril. 84, 833–842.

García-Contreras, A., De Loera, J., García-Artiga, C., Palomo, A., Guevara, J.A., Herrera-Haro, J., López-Fernández, C., Johnston, S., Gosálvez, J., 2011. Elevated dietary intake of Zn-methionate is associated with increased sperm DNA fragmentation in the boar. Reprod. Toxicol. 31, 570–573.

García-Peiró, A., Martínez-Heredia, J., Oliver-Bonet, M., Abad, C., Amengual, M.J., Navarro, J., Jones, C., Coward, K., Gosálvez, J., Benet, J., 2011. Protamine 1 to protamine 2 ratio correlates with dynamic aspects of DNA fragmentation in human sperm. Fertil. Steril. 95, 105–109.

Gawecka, J.E., Marh, J., Ortega, M., Yamauchi, Y., Ward, M.A., Ward, W.S., 2013. Mouse zygotes respond to severe sperm DNA damage by delaying paternal DNA replication and embryonic development. PLoS ONE 8, e56385.

Gosálvez, J., López-Fernández, C., Fernández, J.L., Gouraud, A., Holt, W.V., 2011a. Relationships between the dynamics of iatrogenic DNA damage and genomic design in mammalian spermatozoa from eleven species. Mol. Reprod. Dev. 78, 951–961.

Gosálvez, J., Núñez, R., Fernández, J.L., López-Fernández, C., Caballero, P., 2011b. Dynamics of sperm DNA damage in fresh versus frozen-thawed and gradient processed ejaculates in human donors. Andrologia 43, 373–377.

Guerif, F., Lemseffer, M., Leger, J., Bidault, R., Cadoret, V., Chavez, C., Gasnier, O., Saussereau, M.H., Royere, D., 2010. Does early morphology provide additional selection power to blastocyst selection for transfer. Reprod. Biomed. Online 21, 510–519.

Jędrzejczak, P., Taszarek-Hauke, G., Derwich, K., Depa, M., Pawelczyk, L., 2004. The effect of selected sperm characteristics on their fertilizing potential during IVF procedures. Ginekol. Pol. 75, 946–955.

Jiang, H.H., He, X.J., Song, B., Cao, Y.X., 2011. Sperm chromatin integrity test for predicting the outcomes of IVF and ICSI. Zhonghua Nan Ke Xue. 17, 1083–1086.

Perrault, S.D., Aitken, R.J., Baker, H.W.G., Evenson, D.P., Huszar, G., Irvine, D.S., Morris, I.D., Morris, R.A., Robbins, W.A., Sakkas, D., Spano, M., Wyrobek, A.J., 2003. Integrating new tests of sperm genetic integrity into semen analysis: breakout group discussion. In: Robaire, B., Hales, B.F. (Eds.), Advanced in Male Mediated Developmental Toxicity. Kluwer Academic/Plenum Publishers, New York, pp. 253–268.

Pregl Breznik, B., Kovačič, B., Vlaisavljević, V., 2013. Are sperm DNA fragmentation. hyperactivation. and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? Fertil. Steril. 99, 1233–1241.

Pribenszky, C., Losonczi, E., Molnar, M., Lang, Z., Mátyás, S., Rajczy, K., Molnár, K., Kovács, P., Nagy, P., Conceicao, J., Vajta, G., 2010. Prediction of in-vitro development competence of early cleavage-stage mouse embryos with compact time-lapse equipment. Reprod. Biomed. Online 20, 371–379.

Rahman, M.B., Kamal, M.M., Rijsselaere, T., Vandaele, L., Shamsuddin, M., Van Soom, A., 2014. Altered chromatin condensation of heat-stressed spermatozoa perturbs the dynamics of DNA methylation reprogramming in the paternal genome after in vitro fertilisation in cattle. Reprod. Fertil. Dev. 26, 1107–1116.

Rougier, N., Uriondo, H., Papier, S., Checa, M.A., Sueldo, C., Alvarez Sedó, C., 2013. Changes in DNA fragmentation during sperm preparation for intracytoplasmic sperm injection over time. Fertil. Steril. 100, 69–74.

Sadeghi, M.R., Lakpour, N., Heidari-Vala, H., Hodjat, M., Amirjannati, N., Hossaini Jadda, H., Binaafar, S., Akhondi, M. M., 2011. Relationship between sperm chromatin status and ICSI outcome in men with obstructive azoospermia and unexplained infertile normozoospermia. Rom. J. Morphol. Embryol. 52, 645–651.

Santiso, R., Tamayo, M., Gosálvez, J., Johnston, S., Mariño, A., Fernández, C., Losada, C., Fernández, J.L., 2012. DNA fragmentation dynamics allows the assessment of cryptic sperm damage in human: evaluation of exposure to ionizing radiation. hyperthermia. acidic pH and nitric oxide. Mutat. Res. 734, 41–49.

Simon, L., Murphy, K., Shamsi, M.B., Liu, L., Emery, B., Aston, K.I., Hotaling, J., Carrell, D.T., 2014. Paternal influence of sperm DNA integrity on early embryonic development. Hum. Reprod. 29, 2402–2412.

Tremellen, K., 2008. Oxidative stress and male infertility – a clinical perspective. Hum. Reprod. Update 14, 243–258.

Wale, P.L., Gardner, D.K., 2010. Time-lapse analysis of mouse embryo development in oxygen gradient. Reprod. Biomed. Online 21, 402–410.